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FEASIBILITY STUDY OF AN EXPERIMENT TO MEASURE THE RBE OF TRITIUM FOR THE INDUCTION OF MYELOID LEUKEMIA IN ANIMALS

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ABSTRACT

A variety of RBE values ranging from about 1 to 3 for tritium have been measured by different investigators. The reason for this range in RBE can be attributed to differences in the biological endpoints measured, the reference radiation to which the effects of tritium were compared, and the tritium dosimetry of the particular study. Since the principal risk of low-level irradiation is the induction of cancers, it would be desirable to utilize this endpoint in tritium RBE experiments if these experiments are to be used to evaluate the quality factor for tritium. Furthermore, it would be desirable to use 200 kVp X-rays as the reference radiation since this radiation was suggested by ICRP as the standard reference to be used in the calculation of dose equivalents for purposes of radiation protection. Acute myeloid leukemia is one of the earliest recognized examples of radiogenic cancer in humans and this endpoint has also been the subject of animal studies.

This report gives the results of a review of these animal studies to see if this endpoint is suitable for an experiment to measure the tritium RBE relative to 200 kVp X-rays. It was concluded that the male CBA/H mouse, would be a suitable species and an experiment involving 5000 animals in a four to five year study would be required to provide a useful estimate of the RBE for tritium.

RÉSUMÉ

Différents chercheurs ont relevé plusieurs valeurs variant entre 1 et 3 pour l'efficacité biologique du tritium. On attribue ces variations aux différences des points d'arrêt biologiques qui ont été mesurés, aux rayonnements de référence auxquels on a comparé les effets du tritium et à la dosimétrie relative au tritium utilisée pour l'étude en particulier. Puisque le risque principal d'une faible irradiation est de provoquer le cancer, il serait désirable d'utiliser ce point d'arrêt dans les expériences pour déterminer l'efficacité biologique relative du tritium, si l'on veut se servir de ces expériences pour évaluer le facteur de qualité du tritium. En outre, il serait désirable d'utiliser des rayons X de 200 kVp comme rayonnements de référence, tel que suggéré par la CIPR, pour calculer les équivalents de dose en radioprotection. La leucémie myéloïde aiguë est l'un des exemples reconnus le plus précoces de cancer radiogénique chez l'être humain et ce point d'arrêt a également fait l'objet d'études sur des animaux.

Le présent rapport donne les résultats de l'examen de ces études sur des animaux pour vérifier si ce point d'arrêt convient pour mesurer l'efficacité biologique relative du tritium par rapport à des rayons X de 200 kVp. Le rapport conclut que la souris mâle CBA/H représenterait une espèce convenable et qu'il faudrait une expérience portant sur 5000 animaux au cours d'une étude étalée sur une période de quatre à cinq ans pour fournir un calcul estimatif utile de l'efficacité biologique relative du tritium.

TABLE OF CONTENTS

ABSTRACT		i
1.	INTRODUCTION	1
2.	LITERATURE REVIEW OF ANIMAL STUDIES	3
3.	DERIVATION OF A STATISTICAL SIGNIFICANT EXPERIMENT SIZE	6
4.	EXPERIMENTAL REQUIREMENTS	9
5.	SUMMARY AND CONCLUSIONS	12
REFERENCES		14
TABLES		22

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1. INTRODUCTION

A variety of RBE values ranging from about 1 to 3 for tritium have been measured by different investigators. The reason for this range in RBE can be attributed to differences in the biological endpoints measured, the reference radiation to which the effects of tritium were compared, and the tritium dosimetry of the particular study. Because of this range of results, and because the assumed general relationship[1] between LET and radiation quality predicts a value (correctly or not) of 1.7 for tritium, the quality factor (Q) used to convert absorbed dose (Gy) from tritium exposures to dose equivalent (Sv) ranges from 1 to 2 in different countries.

Since the principal risk of low-level irradiation is the induction of cancers, it would be desirable to utilize this endpoint in tritium RBE experiments if these experiments are to be used to evaluate the quality factor for tritium. Furthermore, it would be desirable to use 200 kVp X-rays as the reference radiation since this radiation was suggested by ICRP[1, 2] as the standard reference to be used in the calculation of dose equivalents for purposes of radiation protection.

There has been only one large scale experiment to date that evaluated tritium RBE which incorporated a carcinogenic endpoint with 200 kVp X-rays as the reference radiation. This study[3] used the acceleration of the appearance of mammary tumors in female Sprague-Dawley rats as the carcinogenic endpoint. The RBE measured was approximately 1.2, but could not be reliably distinguished from 1.0. While the results of this study are useful in furthering our knowledge of the carcinogenic effects of tritium as compared to other radiation types, it is desirable to have results from experiments that use the induction of cancer as an endpoint, rather than the acceleration of its appearance. It would also be desirable to study a cancer type which can be induced by radiation in humans.

Acute myeloid leukemia, one of the earliest recognized examples of radiogenic cancer, has a significantly higher incidence in people exposed to whole body irradiation as compared to unexposed persons[4-6]. This endpoint has also been the subject of animal studies where animals were exposed to external irradiation. The relationship between incidence and external radiation dose is thought to have a linear component and is seemingly without threshold[4,7-15]. There are also a limited number of animal experiments reported that deal with leukemia induction from incorporated radionuclides (e.g. Sr-90, I-131, P-32, etc.)[7,8,16,18-21]. It appears that the leukemia incidence in these experiments is linearly related to the average dose to the hematopoeitic stem cells' nuclei. However, there are no reported experimental data assessing this endpoint for tritium.

Tritium is of increasing importance in nuclear power production, particularly in Canada because of the commitment to CANDU (heavy water) reactors, but also in other countries studying fuel reprocessing or fusion reactors. It would therefore seem reasonable to consider a study of the RBE for tritium induced myeloid leukemia in animals. This report outlines the feasibility of such a study.

2. LITERATURE REVIEW OF ANIMAL STUDIES

Leukemias are classified clinically on the basis of the duration of character of the disease (acute or chronic) and the type of cell involved (myeloid or myelogenous, lymphoid or lymphogenous, reticular, etc.) (5,21,22).

Most radiation-induced leukemias in man are acute or chronic myelogenous (granulocytic) and none are of the chronic lymphatic type[11-14]. A summary of the types of leukemias studied in various animal species is given in Table 1.

In order for an animal species to serve as an appropriate model for tritum beta-ray induced leukemia in man it would need to exhibit a specific sensitivity toward radiogenic myeloid leukemia, rather than a non-myeloid type leukemia. Though myeloid leukemia has been observed in monkeys, dogs, cats and swine exposed to Sr-90[16] the expense of maintenance of these larger animals limits the number of animals in an experiment, and hence limits the statistical significance of any results of the experiment. Statistically significant tritium RBE experiments can only be done at reasonable cost with rodents.

In rodents, the effects of radiation on the incidence of myeloid leukemia are rather inconsistent in the rat but apparently consistent in the mouse model[23]. While thymic lymphoma, reticular cell sarcoma and lymphosarcoma found in mice have no clear human counterpart, myeloid leukemia in mice shows some similarity with human myeloid leukemia as far as pathology, sex and dose response are concerned[5,11,24]. One consideration of the mouse model which must be considered however, is that viral activation has been implicated in myeloid leukemia induction in many strains. A number of investigators[11, 24-38] have isolated cell-free leukemogenic agents from radiation-induced leukemias in mice. These agents appear to be endogenous viruses activated and/or released by radiation, which upon interaction with susceptible host cells results in malignant cell transformations[26,36,38]. Though most strains of mice are thought to harbour the endogenous viral material in their genome, there is considerable variability of viral expression in leukemogenesis from strain to strain[32-34]. The expression of leukemia viruses is controlled by the genome of the host, with each mouse strain showing its own age-related spectrum of viral expression. Consequently, mouse strains have been classified as:

- high virus strains: positive for viral activity throughout life
- low virus strains: positive for viral activity only during part of life
- non virus strains - 1) absence of all or part of virus
  or 2) virus is present but not expressed at any stage in life

Table 2 lists the various mouse strains according to viral expression.

The role of viruses in human myeloid leukemia is uncertain. Though there have been sporadic reports of filterable agents (presumably viruses) from some leukemic patients, in the majority of cases no relationship to viral activation has been established[5,6,39].

From the above considerations a suitable mouse model to carry out a tritium RBE experiment would be one that:

1) was sensitive to radiation produced myeloid leukemia
and
2) had minimal viral involvement

Referring to Table 2 it would seem that of the mouse strains studied, the CBA mouse best fulfills these requirements. Exposure of these mice to radiation increases the incidence of myeloid leukemia with little effect on the low spontaneous incidence of other types of leukemia.

Radiogenic myeloid leukemia is more readily induced in the male mouse than in the female, apparently due to the inhibitory effect of estrogens (3,15,27,39,40). For this reason most work has been done on the male mouse. The dose-response for acute (0.042 to 5.5 Gy/min) x-irradiation on the incidence of myeloid leukemia in the male CBA mouse was found to increase up to about 3 Gy and then decrease[42-47] (see Table 3).

The spontaneous incidence for myeloid leukemia in the male CBA mouse is apparently 0% (0 cases in 800 control mice[47]) whereas the maximum acute radiation induced incidence is about 20 to 25% at a dose of 3 to 4 Gy. [By way of contrast, the lifetime risk of leukemia in humans is about 1.2 to 1.5% after an exposure to 3 Gy of X or γ rays at high dose rates, and is thought to be about 0.2% per Gy at low dose rates (12, 13). The spontaneous lifetime incidence of myeloid leukemia in humans is about 0.3% and is about 0.7% for all leukemias[12,13]]. Table 3 gives the incidence for myeloid leukemia in male CBA mice following various doses of Co-60 gamma rays (Table 3 (a)) and x-rays (Table 3 (b)) at different dose rates.

Apparently, a reduction in dose rate from above 40 mGy per min to 100 μGy per min results in about a 3 to 4 fold reduction in the myeloid leukemia incidence. Tritium has a half-life in the mouse of 1 to 3 days. Therefore a single injection of tritiated water (HTO) to achieve a total dose of 3 Gy would require an average dose rate of about 700 μGy per min, and smaller dose rates at later times. It is apparent therefore that the maximum dose rate one could achieve with HTO would be in the region where an incidence of about 2% per Gy was observed for Co-60 (see Table 3 (a)). It also appears that for low dose rates the peak incidence is about 6% at 3 Gy, although the data at low dose rates is not good enough to state with certainty that the incidence decreases at total doses above 3 to 4 Gy. It is clear that at high dose rates this is the case.

It therefore appears that with our present knowledge, an experiment to measure the RBE of tritium for myeloid leukemia induction would be best carried out on CBA/H mice, and the incidence of induction used for the experimental design should be 2% per Gy. This incidence rate is used to calculate the number of mice required to carry out a statistically significant experiment, as described in Section 3.

3. DERIVATION OF A STATISTICAL SIGNIFICANT EXPERIMENTAL SIZE

The experimental design used in this section is the irradiation of CBA/H mice with X-rays and tritium oxide (HTO) at 1.0, 2.0 and 3.0 Gy at an average dose rate of about 0.5 mGy min^{-1}. The incidence of myeloid leukemia as a function of dose for these mice will then be fitted to a straight line. That is:

$$I_X = A_X + B_X \text{ Dose}$$
$$I_T = A_T + B_T \text{ Dose}$$

where I_X = incidence

A_X = zero dose intercept

B_T = slope of line

and the subscripts X and T refer to X-ray results and tritium results, respectively.

The RBE that will be calculated is given by:

$$RBE = \frac{B_T}{B_X} \pm \frac{B_T}{B_X} \left[\frac{\Delta B_T^2}{B_T^2} + \frac{\Delta B_X^2}{B_X^2} \right]^{1/2}$$

$$= \frac{B_T}{B_X} \pm \Delta RBE \qquad 3.1$$

The value of the standard deviation, ΔRBE, will be a function of the number of mice with myeloid leukemia at each data point. This value can be calculated if one assumes a mean incidence rate (number per Gy) and a probability distribution about the mean incidence rate.

The assumptions used here are that the incidence rate for X-rays and tritium are identical and equal to 2 per 100 animals per Gy, and that the distribution about the mean value at the dose points has a Poisson probability distribution. Once these assumptions have been made, one can choose an experimental design (number of animals at each dose point), calculate a probability distribution for the RBE for each experimental design, and from this procedure estimate the number of animals required to demonstrate that the RBE for tritium is within a given interval with any required confidence.

The calculational procedure used in this work to obtain the distribution of RBE was essentially a monte carlo approach. The number of animals with myeloid leukemia at each dose point following X-ray irradiation was estimated using a random number generator, the assumed distribution, the number of animals, and the assumed incidence rate. This data was used to calculate A_X and B_X. This procedure was repeated for tritium, and the value of A_T and B_T calculated. The value of RBE was then calculated and the process repeated 100 times for each experimental design considered. The results of this process are given in Table 4.

With reference to Table 4, it can be seen that there is about a 15% chance that the measured RBE will be above 1.5 if 2000 mice are used (Column (i)), and a 30% chance that values above 2 could not be excluded at approximately the 95% (RBE + 2ΔRBE) confidence level. It should be emphasized that these results are for an assumed RBE of unity. If one uses 5000 mice, the corresponding results (Table 4, column (iii)), indicate that there is only a 1% chance that the RBE will be above 1.5, and only a 5% chance that an RBE of 2 cannot be excluded.

Since the RBE predicted using the standard LET relationship[1], or more sophisticated microdosimetry relationships (e.g. 48) is between 1 and 2, it is important to design the experiment to insure that the results will be of some use in distinguishing between 1 and 2. It therefore appears that an experiment involving at least 5000 mice is required.

4. EXPERIMENTAL REQUIREMENTS

This section outlines the requirements to conduct a tritium RBE experiment with 5000 male CBA/H mice, as outlined above. It is stressed that this section does not constitute a formal cost analyses, nor should it be considered to be a part of any proposal. It is produced solely to aid in the evaluation of the feasibility of carrying out such an experiment.

The experiment would require approximately five years to complete. The first year would involve breeding a mouse colony up to the required size and carrying out the irradiations. The next three years would consist of maintaining the 5000 mice and examining each animal for myeloid leukemia as it becomes moribund. The final year would involve data reduction and analysis, report writing, etc. The requirements are broken down by year for convenience. Costs for materials and supplies are estimated in 1984 dollars. Costs for labour are estimated in technical (TPY) and professional (PPY) person-years.

Year One

1. Breeding

CBA/H mice are not readily available. Hence a program to breed up a colony large enough to produce 5000 male mice over a short time period would be required. Several options are available.

- All breeding done commercially
  - Cost estimate @ $10/mouse — $50,000

- All breeding done by experimenter
  - Cost of litter and food — 4,000
  - Technical time — 1 TPY
  - Professional time — 0.1 PPY

- Colony bred up commercially and final breeding done by experimenter. (This option would be more suitable than the full commercial one for logistic and technical reasons.)

Animal Cost (@ $8/mouse)	$10,000
Cost of litter and food	500
Technical time	0.1 TPY
Professional time	0.05 PPY

2. Irradiations

This requires a suitable X-ray facility and a tritium handling facility. Animals would have to be maintained in a radioactive laboratory until tritium concentrations decreased to a suitable level. Actual levels would be critically dependent on the type and licence of the laboratory carrying out the work. (Note that tritium requirements will be about 10^8 Bq (2.7 mCi) per mouse per Gy.)

Irradiations themselves will require expertise in external and tritium dosimetry.

Technical time (2 technicians for 5 weeks)	0.2 TPY
Professional time	0.1 PPY
Materials and Supplies	$ 5,000

Year Two

Animal Maintenance

Food and litter	$ 5,000
Technical time	1 TPY
Professional time	0.1 PPY

Scientific (Tissue Analysis, etc.)

Materials and Supplies	$ 2,000
Technical time	0.2 TPY
Professional time	0.2 PPY

Year Three

Animal Maintenance	
Food and Litter	$ 5,000
Technical time	1 TPY
Professional time	0.1 PPY
Scientific (Tissue Analysis, etc.)	
Materials and Supplies	$ 2,000
Technical time	0.2 TPY
Professional time	0.3 PPY

Year Four

Animal Maintenance	
Food and Litter	$ 5,000
Technical time	1 TPY
Professional time	0.1 PPY
Scientific (Tissue Analysis, etc.)	
Materials and Supplies	$ 5,000
Technical time	1 TPY*
Professional time	0.5 PPY*

Year Five

Data reduction and analyses	
Technical time	0.1 TPY**
Professional time	0.25 PPY**
Report writing, etc.	0.25 PPY**

* During peak periods, more than one technical and one professional person may be required to carry out all the analyses required.

** People from several areas of expertise will be required.

In summary, the approximate experimental requirements over the five year study are:

Materials and Supplies (Using third option on breeding mice)	$ 40,000
Technical	4.8 TPY
Professional (Highly qualified specialists are required)	1.95 PPY

Facilities

- Suitable animal housing for 5000 mice for 3 years (625 cages at 8 mice per cage)
- Suitable X-ray (200 kVp) Facility
- Suitable tritium laboratory (approximately 2×10^{11} Bq of tritium to be administered and disposed of in a period of about 5 weeks).
- Suitable facilities for tissue analyses, blood work, etc.

5. SUMMARY AND CONCLUSIONS

The available information on radiation induced myeloid leukemia in animals has been reviewed (Section 2) and it is concluded that with our current knowledge the only suitable species to use in a tritium RBE experiment is the male CBA/H mouse.

It is estimated that this strain has a myeloid leukemia incidence of 2% per Gy for low dose rate irradiations. Using this incidence rate, at least 5000 mice would be required to provide a useful estimate of the RBE for tritium for this end point (Section 3). Any significantly smaller experiment size would not add appreciably to our current understanding of the risk of doses from tritium as compared to those from other sources of ionizing radiation.

Experimental requirements have been estimated (Section 4). The main requirements of this experiment that are unique compared to many other animal experiments is the irradiation (X-ray and tritium) facilities and the radiation dosimetry expertise.

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TABLE 1

Types of leukemia in various species, as reported in the literature. Leukemia types have been grouped into three general categories: M = myeloid leukemia, L = lymphoid leukemia and R = reticuloendothelial sarcomas with or without leukemia.

SPECIES	LEUKEMIA TYPES	VIRAL IMPLICATIONS	REFERENCES
Man	M, L	uncertain	23,4,11,5,39,12,42, 43,13,14,6
Monkeys	M, L	uncertain	5,6
Cattle	L, M	yes	49,6
Swine	M, L	uncertain	16,23
Dog	L,R,M(rare)	uncertain	16,23,49,22,50
Cat	L, R, M	yes	49,22,6
Fowl	M, L	yes	49,6
Rats	M, L	uncertain probably not	23,51,52,53,54,55, 56,57
Mice	see Table 2	see Table 2	see Table 2

TABLE 2

Types of leukemia in various strains of mice, as reported in the literature. Leukemia types as per Table 1.

VIRAL ACTIVITY	MOUSE STRAIN	LEUKEMIA TYPE	REFERENCES
High Virus	AKR/J	L	26,29,30,32,33,34,36,38
	RF	M, L, R	26,40,27,23,24,31,11,58, 59,60,61,41,62,63,64,65, 66,67,15
	C3H/FgLw	L	32,34,68
	C58	L	29,32,38
Low Virus	Balb/c	L, R	26,29,32,34,69,58,60, 62,15
	C57Bl/6J	L	25,26,70,23,29,34,11, 69,15
	C3H/HeN	M, L	26,71,29,34,72
	DBA/2N	L	29,34
	ICR	M, L	19
	LAF1	L	71
	RAP	M, L, R	71
	NZB/N	L, R	34,68
Non Virus	CBA/H	M, L	42,43,68,45,46,47,15,18
	NIH SWISS	L, R	26,32,33,34,37
	C57L/J	L	34
	129/J	L	32,34

TABLE 3

Incidence (%) of myeloid leukemia in CBA/H mice following Co-60 (a) and X-ray (b) irradiation as a function of dose and dose rate. Numbers in brackets following each incidence is the number of mice in each group.

(a) Co-60[47]

DOSE (Gy)	DOSE RATE (Gy min^{-1})		
	4-11 x 10^{-5}	0.25*	0.25
1.5	4[71]	6[72]	11[99]
3.0	6[66]	6[65]	17[83]
4.5	6[66]	5[65]	25[104]

* 20 fractions on a 5 day per week schedule (4 weeks)

(b) 250 kVp (1.1 mm HVL Cu) irradiation[43]

DOSE (Gy)	DOSE RATE (Gy min^{-1})		
	0.042	0.573	5.52
0.75	11[53]	2[52]	12[49]
1.5	10[60]	19[47]	29[48]
3.0	20[40]	25[40]	23[39]
4.5	5[42]	12[42]	14[42]
6.0	2[42]	0[39]	2[41]

TABLE 4

Percent of results predicted to be in the indicated ranges for RBE and the RBE + 2ΔRBE upper limit on RBE, for three experimental designs, calculated on the assumption that the true RBE is close to 1.0.

(a) Percent RBE in indicated ranges

RBE RANGE	PERCENT IN RANGE		
	(i)	(ii)	(iii)
0 - 0.5	2	1	0
0.5 - 0.75	29	8	5
0.75 - 1.0	33	48	50
1.0 - 1.25	21	24	31
1.25 - 1.5	10	16	13
1.5 - 1.75	10	3	1
1.75 -	4	0	0

(b) Percent RBE + 2ΔRBE in indicated ranges

RBE + 2ΔRBE RANGE	PERCENT IN RANGE		
	(i)	(ii)	(iii)
0 - 0.5	0	0	0
0.5 - 1.0	12	8	6
1.0 - 1.5	30	45	61
1.5 - 2.0	29	35	28
2.0 - 2.5	14	10	5
2.5 -	15	2	0

(i) 500 controls, 250 mice at each dose point, total mice = 2000

(ii) 500 controls, 500 mice at each dose point, total mice = 3500

(iii) 500 controls, 750 mice at each dose point, total mice = 5000